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Gametogenesis and Embryonic Development in the Calcareous Sponges *Clathrina coriacea* and *C. blanca* from Santa Catalina Island, California

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Abstract.—Gametogenesis and embryonic development of *Clathrina coriacea* and *C. blanca*, two closely related calcareous sponges from Santa Catalina Island, California, are described. Oogenesis is asynchronous in both species. Spermatogenesis was not observed. Cleavage is total and equal, resulting in the formation of a blastula larva. The larva of *C. coriacea* contains one large posterior granular cell, whereas two posterior granular cells are present in the larva of *C. blanca*. Migration of the larval blastomeres into the blastocoel begins while the larva is in the tube of the parent sponge.

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The life histories of *Clathrina coriacea* (Montagu) and *C. blanca* (Miklucho-Maclay), two closely related calcareous sponges from Santa Catalina Island, California, were studied to clarify their systematic affinity (Johnson, 1976). Minchin (1900), Topsent (1936) and Borojević (1967), among others, treated *C. coriacea* and *C. blanca* as separate species. Burton (1963), on the other hand, believed that the two species names were synonymous.

Lévi (1956) utilized differences in embryological development and breeding period to separate two morphologically identical sponges of the genus *Halisarca* into *H. dujardini* and *H. metschnikovi*. This paper reports the findings of a comparative study of gametogenesis and embryonic development in *C. coriacea* and *C. blanca*.

Methods

Clathrina coriacea and *C. blanca* were studied at Santa Catalina Island from April 1973 through October 1975. The sponges were collected at weekly intervals during the reproductive period in order to obtain detailed information on gametogenesis and embryonic development. Most of the specimens were obtained from a submarine grotto and the Santa Catalina Marine Biological Laboratory pier in Big Fishermans Cove.

Collection of the sponges involved carefully removing them from the attachment surface with forceps and placing them in labelled plastic bags. The specimens were immediately transferred to sea water Bouin's Fixative for preservation of the sponge and decalcification of the spicules. The spicules were dissolved to facilitate observation of the reproductive structures and histological procedures. After decalcification was complete (24 to 48 hours) the sponges were transferred to 70% ethanol. They were then observed under a dissecting microscope for the presence of oocytes. Observations of the location of the oocytes within each sponge also were recorded.

A small piece of each sponge, or the whole sponge when it was less than 5 mm in size, was used for histological study. The sponges were dehydrated, stained temporarily with a weak solution of Fast Green in 70% ethanol, embedded in Paraplast (Sherwood Medical Industries) and sectioned at 8 μm. Every fifth or tenth section, depending on the size of the embedded piece, was mounted on a glass slide with a minimum of 30 sections from each sponge. Minute specimens were sectioned serially. The slides were stained with Ehrlich's Hematoxylin and counterstained with Eosin.

Results

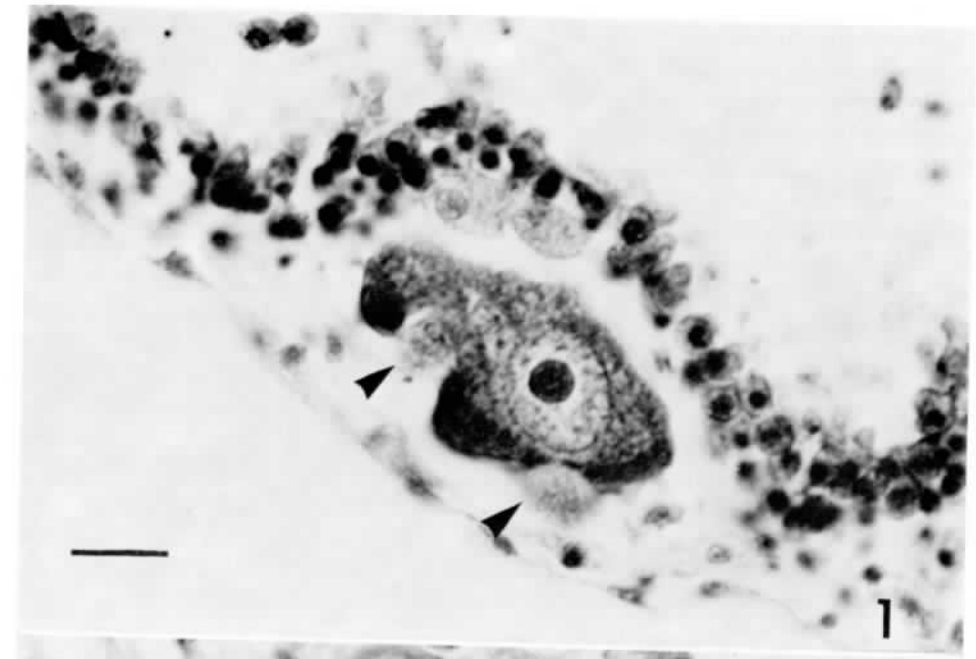
Reproductive Season.—Maturing oocytes were observed in *C. blanca* between April and June in 1973 and 1974, and between April and August in 1975. In *C. coriacea* reproductive elements were seen from July to September in 1973, from July to August in 1974 (specimens were not collected during September and October), and from July through October in 1975 (Johnson, 1978).

Oogenesis.—The process of oocyte development is similar in *C. coriacea* and *C. blanca*. The young oocyte can be distinguished from other nucleolate cells by its strongly basophilic staining cytoplasm, large nucleus and nucleolus surrounded by a deeply staining nuclear membrane. As the oocyte grows it begins to push the surrounding layer of choanocytes into the tube of the parent sponge. Oocytes larger than 30 μm apparently phagocytize the surrounding eosinophilic amebocytes (Fig. 1). These amebocytes are common in specimens with developing oocytes, but rare in nonreproducing sponges. The length of the eosinophilic cells varies from 7 to 10 μm in *C. coriacea* and *C. blanca*. Although there is no distinction in the dimensions of the eosinophilic amebocytes between the two species, noticeable differences exist in the eosinophilic granules. In *C. coriacea* the eosinophilic amebocytes are filled with numerous highly refractile eosinophilic staining granules. In *C. blanca*, on the other hand, the granules are smaller, less refractile and less abundant. Continued growth of the oocyte results in a cell highly granular in appearance (Fig. 2). The maximum length recorded for a mature oocyte of *C. coriacea* was 90 μm. Oocytes 100 μm in length were seen in *C. blanca*.

There is no apparent synchronization of oogenesis within the breeding population of the two species. Some of the specimens collected at the same time contain no oocytes, some have large oocytes and others contain embryos or larvae. Occasionally small and large oocytes, oocytes and embryos or larvae occur in the same sponge. The different stages of development are generally found in different regions of the sponge.

Spermatogenesis.—All of the specimens collected during the reproductive season were examined for stages of spermatogenesis. No sperm or spermatic cysts were seen in the two species.

Fertilization.—The process of sperm transport to the oocyte was not observed with certainty in *C. coriacea* or *C. blanca*. Occasionally a small deeply staining ovoid structure enclosed within a vesicle was seen in a choanocyte near the oocyte. It resembled Tuzet's figure of a spermatozoan in the choanocyte (1947:plate 1, fig. 17). Some cells between the choanocyte layer and the oocyte appeared to contain two nuclei. According to Tuzet (1947:plate 1, fig. 13), one of the "nuclei" is actually a spermatic vesicle in the cytoplasm of a carrier cell. On



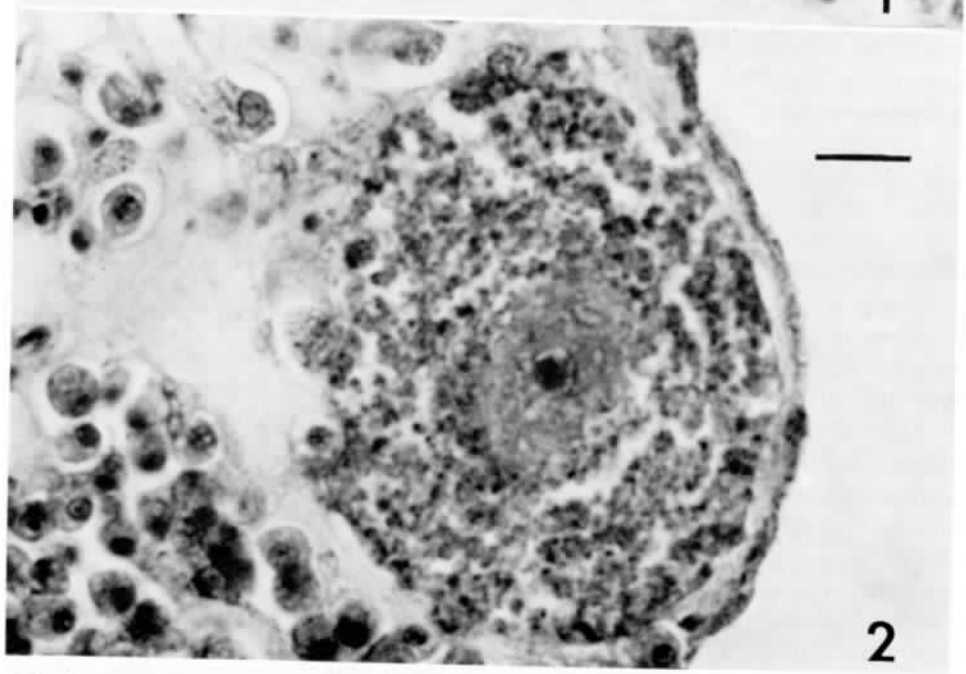
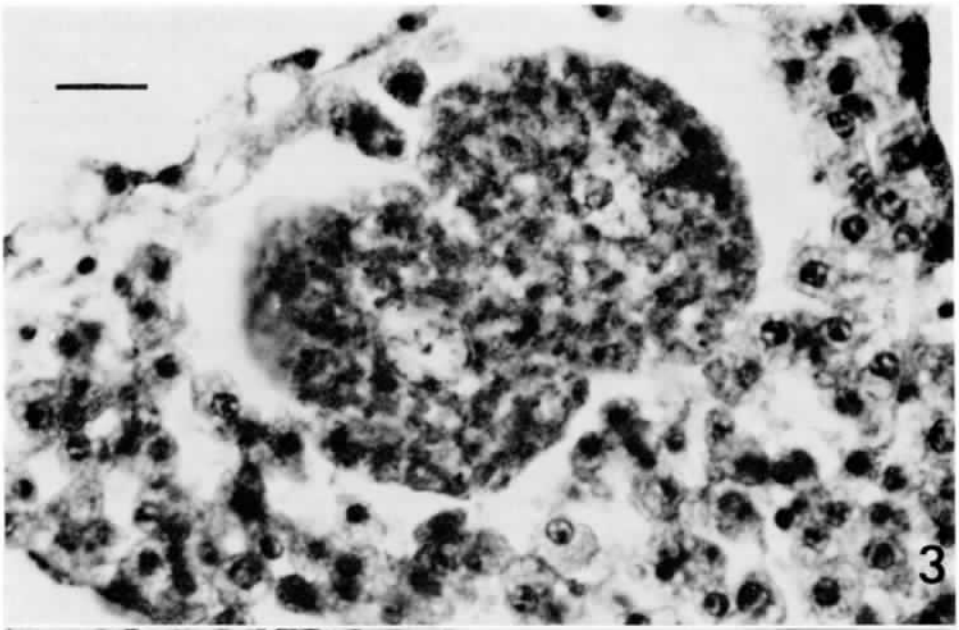


Fig. 1. Oocyte of *Clathrina blanca* phagocytizing eosinophilic amebocytes (arrows). Scale, 10 μm.

Fig. 2. Large granular oocyte of *Clathrina coriacea*. Scale, 10 μm.

rare occasions an oocyte contained two deeply staining masses within a vesicle. These structures resembled Tuzet's figure of the spermatozoan within its vesicle at the periphery of the oocyte (1947:plate 1, fig. 19). Fertilization was not seen in either species.



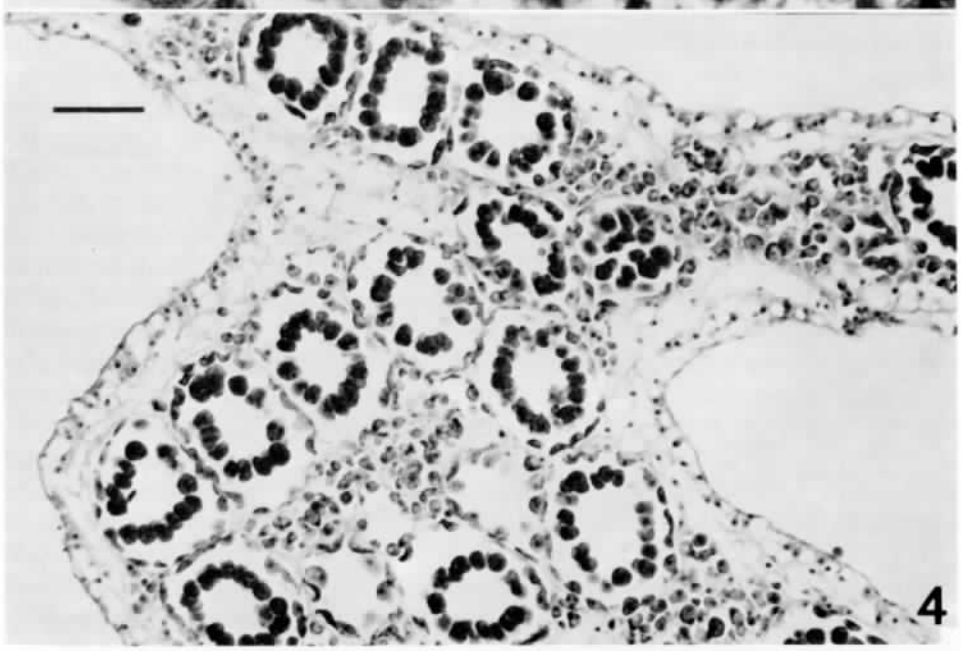


Fig. 3. Two cell stage of cleavage of *Clathrina blanca*; note the nucleus and large nucleolus. Scale, 10 μm.

Fig. 4. Young embryos of *Clathrina coriacea*; note the flattened, elongated cells surrounding each embryo and the unorganized appearance of the parent tissue. Scale, 50 μm.

Embryonic Development.—Cleavage in *C. coriacea* and *C. blanca* is total and equal. The two cell stage, illustrated in Fig. 3, shows the granular cytoplasm, and the nucleus and nucleolus. In the four cell and eight cell stages further equal divisions occur. After the eight cell stage a blastula is formed. The young blastula

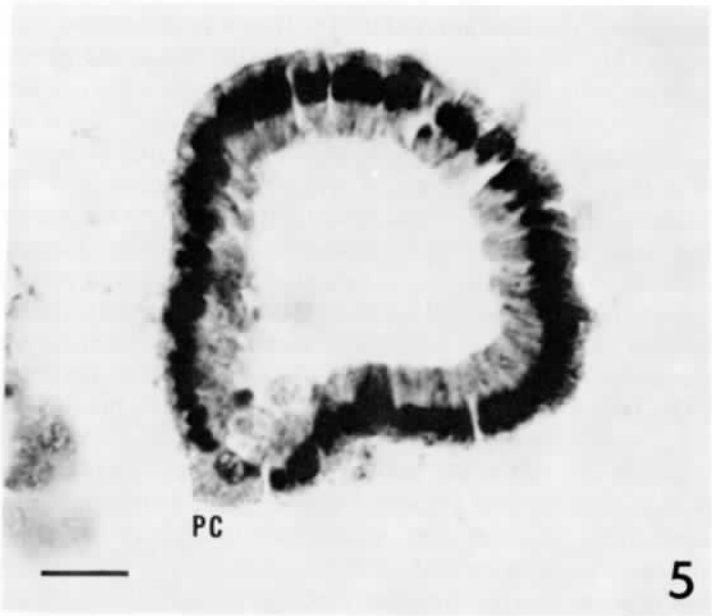
embryo is composed of cells, approximately 12 μm in dimension, surrounding a large blastocoel (Fig. 4). Each embryo is enclosed within the parent tissue by flattened elongate cells. The sponge tissue in which the embryos develop loses its normal appearance and bears little resemblance to that found in specimens without reproductive elements (Fig. 4). The later stages of cleavage take place in the tubes of the parent sponge and result in the formation of a blastula larva. The blastula larva contains two types of cells, the abundant blastomeres and the rare posterior granular cells. The blastomeres are narrow columnar flagellated cells containing deeply staining elongate nuclei located at the periphery of the larva. The posterior granular cell is a large nucleolated cell. The larva of *C. coriacea* contains one large posterior granular cell (Fig. 5), whereas two posterior granular cells are present in the larva of *C. blanca* (Fig. 6). These cells are not seen in all the larvae in a sponge section as it depends on how the larva is sectioned.

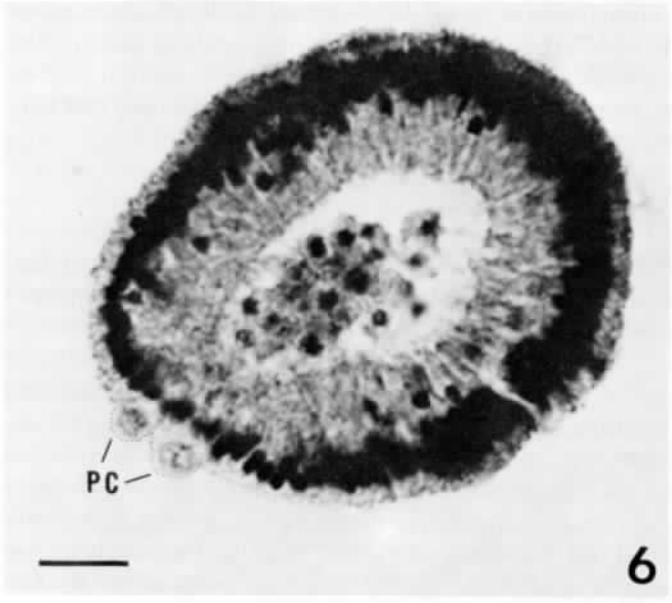
While the larvae are still in the tube of the parent sponge some of the blastomeres begin to migrate into the large blastocoel, losing their flagella and columnar shape and becoming spherical (Fig. 6). The obliteration of the blastocoel progresses at different rates in individual larvae. The blastocoel of some larvae remains relatively free of cells, whereas others are partially or completely filled with cells (Fig. 7). At the completion of larval development the tubes of the parent sponge are solidly packed with larvae moving towards the oscula to be expelled. Measurements of the larvae in the two species reveal much variation in their dimensions. Larvae range from 60 to 150 μm in length in *C. coriacea*, and from 70 to 160 μm in *C. blanca*.

Discussion

Oocytes of *C. coriacea* and *C. blanca* from Santa Catalina Island larger than 30 μm in length appear to phagocytize the eosinophilic amebocytes that aggregate around them. Similar observations were made by Tuzet (1947) in her study of *Leucosolenia coriacea* (=*C. coriacea*). Sarà (1955ac) reported that the oocyte of *C. coriacea* forma *blanca* (=*C. blanca*) first phagocytized choanocytes and then later eosinophilic cells, whereas the oocyte of *C. coriacea* forma *coriacea* (=*C. coriacea*) engulfed only choanocytes. These differences between the two species were not observed in the California specimens. There appears to be no difference in the intensity of phagocytosis between *C. coriacea* and *C. blanca* from Santa Catalina Island. Sarà (1955a), on the other hand, observed that phagocytosis was greater in *C. blanca* than in *C. coriacea*. The eosinophilic amebocytes are rare in nonreproducing individuals from Santa Catalina Island and common in specimens with developing oocytes. Sarà (1955b), however, reported that the eosinophilic amebocytes decreased in specimens with developing oocytes.

The eosinophilic amebocytes differ between the two sponges from Santa Catalina Island. In *C. coriacea* the granules within these amebocytes are larger, more numerous and more refractile than in *C. blanca*. Variations in the eosinophilic amebocytes among species of calcareous sponges also were reported by Minchin (1898), Duboscq and Tuzet (1936) and Borojević (1969). Minchin (1898), in fact, believed that he could distinguish between species of *Clathrina* solely on the basis of the granular (eosinophilic) amebocytes.





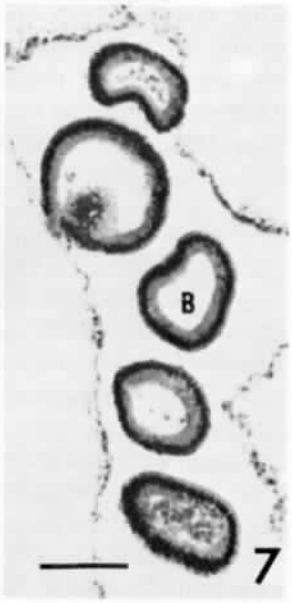


Fig. 5. Single large posterior granular cell (PC) in larva of *Clathrina coriacea*. Scale, 10 μm.
Fig. 6. Two large posterior granular cells (PC) in larva of *Clathrina blanca*. Scale, 10 μm.
Fig. 7. Larvae of *Clathrina blanca*; note the varying stages of migration of the blastomeres into the blastocoel (B). Scale, 50 μm.

The oocyte in *C. coriacea* and *C. blanca* becomes very granular as it reaches sexual maturity. Similar observations were made by Tuzet (1947) for *C. coriacea*.

Oogenesis is asynchronous in the breeding population of the two California sponges. Some specimens contain developing oocytes, embryos or larvae, where-

as other individuals have no reproductive elements. Duboscq and Tuzet (1937) reported that oogenesis also was asynchronous in the calcareous sponge *Grantia compressa*.

Spermatogenesis was not observed in *C. coriacea* or *C. blanca* from Santa Catalina Island. The lack of evidence of stages of spermatogenesis in calcareous sponges has puzzled sponge specialists since the latter part of the nineteenth century. Although Haeckel (1871) described spermatozoa in calcareous sponges, Dendy (1914) wrote that no one else had been able to repeat Haekel's observations. Poléjaeff (1882) collected rare male specimens of *Sycon raphanus* which he said were so completely filled with spermatic cysts that their whole development could be traced in a single section. Görich (1903ab, 1904) reported the presence of spermatic cysts in the upper third of *Sycandra raphanus* (=*Sycon raphanus*) and described the early stages of development. Dendy (1914) described what he thought were spermatic cysts and some stages of spermatogenesis in *Grantia compressa*. Gatenby (1920, 1927) looked at many breeding specimens of *Grantia compressa* and saw only a single stage of spermatogenesis. He concluded that spermatogenesis must take place sporadically and very rapidly. Vacelet (1964) was unable to find stages of spermatogenesis in *Petrobiona massiliana*. Tuzet (1973) reported that spermatogenesis was not known in calcareous sponges. In the demosponges, on the other hand, spermatogenesis has been seen in many species (Tuzet, 1930; Lévi, 1956; Tuzet and Pavans de Ceccatty, 1958; Tuzet and Paris, 1964; Tuzet *et al.*, 1970, among others).

Fertilization was not observed with certainty in *C. coriacea* or *C. blanca* from Santa Catalina Island. Sarà (1955a) and Borojević (1969) also had little success in following the process of fertilization. Borojević (1969) reported that he never saw any figures of fertilization in the numerous specimens of the Calcarea Calcinea he studied, whereas they were easily discernible in the Calcarea Calcaronea. Tuzet (1947), however, described the process of fertilization of *C. coriacea* in great detail, but she did not observe the actual fusion of the male and female pronuclei.

Cleavage in *C. coriacea* and *C. blanca* is total and equal, and a blastocoel forms after the eight cell stage. Similar observations were made by Tuzet (1948) for *C. coriacea* and by Borojević (1969) for other calcinean Calcarea. In the developing blastula larva of *C. coriacea* and *C. blanca* two cell types become apparent, the narrow columnar flagellated cell and the large granular cell. Tuzet (1948) reported that the granular cell determined the posterior region of the larva. Borojević (1969) considered the posterior granular cell to be a blastomere whose division had been retarded. The blastula larva of *C. coriacea* from Santa Catalina Island contains one posterior granular cell, whereas *C. blanca* has two posterior granular cells. These observations agree with Minchin (1900) who reported that *C. coriacea* contains one, *C. blanca* has two, and *C. contorta* and *Ascandra falcata* have four posterior granular cells, whereas *C. cerebrum* and *C. reticulum* do not contain posterior granular cells. In the two California sponges some of the larval blastomeres migrate into the large blastocoel while still within the tubes of the parent sponge. This process also was observed in *Leucosolenia coriacea* (=*C. coriacea*) by Minchin (1896) and Tuzet (1948).

The relationship between the blastula larva of calcareous sponges and other larval types of sponges was discussed by Tuzet (1948, 1973) and Borojević (1969).

Unlike the amphiblastula larva, the blastula larva is formed directly by multiplication of the blastomeres. There is no stage of blastomeres with internal flagella, no inversion of the surfaces, and no formation of the typical "cellules en croix." Borojević (1969, 1970) also found no similarity between the blastula larva and the parenchymella larva of many demosponges. In the parenchymella larva no blastocoel is formed and cellular differentiation occurs very early in the development. The parenchymella larva possesses at its liberation from the parent sponge the principal cell types of the adult sponge, whereas in the blastula larva the larval cells remain totipotent (Tuzet, 1948; Borojević, 1969, 1970).

Conclusion

The differences in the eosinophilic amebocytes, the number of posterior granular cells in the larvae, and the dimensions of the oocytes and larvae, in addition to the differences in the reproductive period between *C. coriacea* and *C. blanca*, reaffirm that the two sponges are separate species.

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